Bacterial epibionts of Daphnia: A potential route for the transfer of dissolved organic carbon in freshwater food webs

Eckert, Ester M ; Pernthaler, Jakob

Abstract: The identification of interacting species and elucidation of their mode of interaction may be crucial to understand ecosystem-level processes. We analysed the activity and identity of bacterial epibionts in cultures of Daphnia galeata and of natural daphnid populations. Epibiotic bacteria incorporated considerable amounts of dissolved organic carbon (DOC), as estimated via uptake of tritiated leucine: three times more tracer was consumed by microbes on a single Daphnia than in 1 ml of lake water. However, there was virtually no incorporation if daphnids were anaesthetised, suggesting that their filtration activity was essential for this process. Microbial DOC uptake could predominantly be assigned to microbes that were located on the filter combs of daphnids, where the passage of water would ensure a continuously high DOC supply. Most of these bacteria were Betaproteobacteria from the genus Limnohabitans. Specifically, we identified a monophyletic cluster harbouring Limnohabitans planktonicus that encompassed sequence types from D. galeata cultures, from the gut of Daphnia magna and from daphnids of Lake Zurich. Our results suggest that the epibiotic growth of bacteria related to Limnohabitans on Daphnia spp. may be a widespread and rather common phenomenon. Moreover, most of the observed DOC flux to Daphnia in fact does not seem to be associated with the crustacean biomass itself but with its epibiotic microflora. The unexplored physical association of daphnids with heterotrophic bacteria may have considerable implications for our understanding of carbon transfer in freshwater food webs, that is, a trophic 'shortcut' between microbial DOC uptake and predation by fish.

DOI: https://doi.org/10.1038/ismej.2014.39

Category: Microbe-microbe and microbe-host interactions

Bacterial epibionts of *Daphnia:* a potential route for the transfer of dissolved organic carbon in freshwater food webs

Short title: Substrate uptake by epibionts of Daphnia

Ester M. Eckert & Jakob Pernthaler*

Limnological Station, Institute of Plant Biology, University of Zurich, Seestrasse 187, 8802 Kilchberg, Switzerland

*corresponding author: Phone: 0041 44 634 9210; Fax: 0041 44 634 92 25; Email: pernthaler@limnol.uzh.ch

Keywords: Daphnia, epibionts, symbiosis, freshwater, food webs, dissolved organic carbon

Abstract

The identification of interacting species and elucidation of their mode of interaction may be crucial to understand ecosystem-level processes. We analysed the activity and identity of bacterial epibionts in cultures of *Daphnia galeata* and of natural daphnid populations. Epibiotic bacteria incorporated considerable amounts of dissolved organic carbon (DOC), as estimated via uptake of tritiated leucine: three times more tracer was consumed by microbes on a single *Daphnia* than in a mL of lake water. However, there was virtually no incorporation if daphnids were anesthetized, suggesting that their filtration activity was essential for this process. Microbial DOC uptake could predominantly be assigned to microbes that were located on the filter combs of daphnids, where the passage of water would ensure a continuously high DOC supply. Most of these bacteria were *Betaproteobacteria* from the genus *Limnohabitans*. Specifically, we identified a monophyletic cluster harbouring *L. planktonicus* that encompassed sequence types from *D. galeata* cultures, from the gut of *D. magna,* and from daphnids of Lake Zurich. Our results suggest that the epibiotic growth of bacteria related to *Limnohabitans* on *Daphnia* spp. may be a widespread and rather common phenomenon. Moreover, most of the observed DOC flux to *Daphnia* in fact does not seem to be associated with the crustacean biomass itself but with its epibiotic microflora. The unexplored physical association of daphnids with heterotrophic bacteria may have considerable implications for our understanding of carbon transfer in freshwater food webs, i.e., a trophic ‘shortcut’ between microbial DOC uptake and predation by fish.

Introduction

In order to accurately estimate element fluxes or predict ecosystem responses it is essential to understand food web architecture (Bascompte, 2010). Unexplored trophic links may considerably alter food web structure, and can, once investigated, substantially change our understanding of ecosystem carbon metabolism (Corno et al, 2012, Kuwae et al, 2012). Therefore, interacting species and their mode of interaction need to be identified for more accurate assumptions about ecosystem functionality and stability (Mougi and Kondoh, 2012, Thébault and Fontaine, 2010).

The lack of information about the physical interactions between heterotrophic bacteria and zooplankton, such as the crustacean genus *Daphnia*, may neglect an important aspect of freshwater food webs (Tang et al, 2010). Indeed, little is known about the microbial communities associated with healthy *Daphnia*, as opposed to the plethora of research about the respective roles of either bacterio- or zooplankton within aquatic food webs, or about microbial parasites of *Daphnia* (e.g. Ebert 2008, Miner et al 2012, Newton et al 2011). Recent studies, however, point to other system-relevant associations between bacteria and *Daphnia,* e.g. the transfer of microbes from lower to higher water layers via attachment-detachment processes (Grossart et al, 2010). The gut microflora of *Daphnia magna* was reported to be dominated by members of the genus *Limnohabitans* (Freese and Schink, 2011), i.e., by common inhabitants of the pelagic zone of freshwater epilimnia that typically co-occur with phytoplankton (Šimek et al, 2005, Šimek et al, 2011). High bacterial diversity, including phylotypes related to *Limnohabitans,* was also found when analysing prokaryotic sequences from metagenomic data of *Daphnia* spp. (Qi et al, 2009).

While algae are generally regarded as the main food for daphnids, heterotrophic bacteria are considered less important (e.g. Martin-Creuzburg et al 2011, Nagata and Okamoto 1988, Peterson et al 1978). However, some bacteria, notably filamentous morphotypes, are also consumed by *Daphnia* sp., which in turn may directly affect bacterial community structure at least during particular seasons (Langenheder and Jürgens, 2001, Pernthaler et al, 2004, Taipale et al, 2008). In addition, it is conceivable that the associations of zooplankton with heterotrophic bacteria may also have substantial implications for biogeochemical processes such as the transfer of carbon through the food web: while daphnids feed on organic

carbon from the particulate fraction (Cole et al, 2006), the attached bacteria would likely consume dissolved organic carbon (DOC). Apart from a single more recent report (Speas and Duffy, 1998), uptake of DOC by *Daphnia* (or by their epibionts) has been addressed by studies dating from the beginning of the last century (e.g. Kerb 1911, Krogh 1930). This largely unexplored trophic link might, however, be of great relevance for lake carbon cycling, e.g., in the context of the much debated question to which extend internal primary production or terrestrial carbon sources support freshwater ecosystems (e.g. Brett et al 2009, Grey et al 2001, Pace et al 2004). In such studies the biomass of homogenised daphnids is proportionally assigned to allochthonous or autochthonous sources -by analysis of the isotopic ratios of carbon atoms- in order to model the fluxes of organic carbon through the food web. The zooplankton epibionts are considered as part of the *Daphnia* biomass; however, their specific metabolic abilities (i.e. consumption of DOC) might considerably affect the interpretation of such assessments. Moreover, epibionts will be consumed by fish together with their host and might, thus, form a shortcut through the food web due to the transfers of organic matter, directly deriving from DOC, to fish.

We studied the uptake of dissolved leucine by *Daphnia galeata* or their epibionts to gain first insight into the importance of this trophic link, and we compared it to their ingestion of leucine incorporating planktonic microbes. Furthermore, we localised and identified a prominent genus of microbial epibionts responsible for this uptake on cultured *D. galeata* as well as on daphnids from mixed natural populations in a lake (Lake Zurich, Switzerland). Using leucine and N-acetyl-D-glucosamine (NAG) as model substrates we then assessed the metabolic activity of *Daphnia* epibionts in Lake Zurich.

Materials and Methods

Sampling and sample preparation

Lake Zurich was sampled weekly at around 10 am between April 26 and May 24 2012 (coordinates 47°31' N, 8°58' E). Chlorophyll *a* (Chl a) and temperature were measured using a multiple-wavelength probe (TS-16–12 fluoroprobe, bbe Moldaenke GmbH, Kronshagen, Germany) and a multi-parameter probe (6600 multi-parameter, water quality monitoring, YSI incorp., Yellow Springs, OH, USA), respectively. The sample taken on April 26 was from the depth of maximum Chl *a* (8m), while later samples were collected from 5m depth because of the onset of the clear water phase (Fig 5). Zooplankton was collected using a Ruttner sampler, concentrated with a 40µm net from a volume of 5L and directly fixed with formaldehyde (FA, final concentration, 4%) for determination of abundances. Live daphnids were collected using the same device and transported to the laboratory in a clean jar. A third set of daphnids were immediately anaesthetised with carbon dioxide enriched water for later experiments. The daphnids were kept in a laboratory incubator at *in situ* temperature for a maximum of 3h prior to the experiments.

Fifty mL of lake water were fixed with FA (final concentration, 1%) for the analysis of total bacterial abundances and the proportions bacteria affiliated with the *Beta-Proteobacteria* genus *Limnohabitans*. Subsamples of 4mL were filtered onto white polycarbonate membrane filters (type GTTP, 45 mm diameter, 0.2 mm pore size, Millipore, Billerica, MA, USA) for the counting of *Limnohabitans* related bacteria. The remaining sample was stored at 4°C for flow cytometric determination of total cell numbers.

Tracer Experiments

In Experiment I (Fig 1) *Daphnia galeata* females were kept in sterile (0.2µm prefiltered and autoclaved) lake water (sLW) and fed with *Scenedesmus subspicatus* approximately every second day. Adult individuals were washed three times with sLW, and 6 sets of 4 individuals were transferred to 50mL Erlenmeyer flasks containing 10mL of sLW. Twelve additional individuals were anaesthetised in commercial carbon dioxide enriched water, washed twice in sLW and 3 sets of 4 individuals were placed in 50mL Erlenmeyer flasks containing 10mL of sLW. Daphnids were acclimatised for 1h in the dark at 20°C before tracer addition.

Nine sets of 30µm prefiltered lake water (10mL each) were incubated for 1h with 10nM of tritium labelled leucine (specific activity: 120 Ci $mmol^{-1}$) or NAG (specific activity: 60 Ci $mmol^{-1}$, American Radiolabeled Chemicals, Inc., St. Louis, Mo, USA) to label the microbial community. Leucine is a widely used marker for biomass production in aquatic microbial ecology (Kirchman et al, 1985). It is incorporated into protein, and there is a large set of data from freshwater systems for comparison (del Giorgio and Cole, 1998, Jørgensen, 1992, Kubitschek, 1968). NAG is the subunit of chitin, the main polymer of the daphnia carapace. Thus, bacteria living on a chitinous surface might arguably have the ability to incorporate NAG (Beier and Bertilsson, 2013, Köllner et al, 2012). The labelled communities were then distributed to triplicate Erlenmeyer flasks containing four live daphnids (treatment Rw) or four anesthetised daphnids (treatment A), respectively. The third set of labelled microbial communities was filtered onto nitrocellulose membrane filters (type GSWP, 45 mm diameter, 0.22µm pore size, Millipore, Billerica, MA, USA) to determine microbial incorporation of the tracer. The filtrate was also collected and added to triplicate Erlenmeyer flasks each containing four daphnids (treatment F).

Live and anesthetised daphnids were incubated for 1h at 20°C on a laboratory rocker (10 rmp over a tilt angle of ±11°). Thereafter, all daphnids were individually picked and anesthetized in carbon dioxide rich water. Three individuals from each Erlenmeyer were then transferred to separate scintillation vials with 500µl Soluene350 (Perkin Elmer Inc., San Jose, CA, USA) to solubilize the tissue and incubated at 50°C for approximately 6h. For one experiment six *D. galeata* individuals were dissected after labelling and colons and the outer carapaces were separated for uptake measurements. When the daphnids were dissolved 0.5mL of scintillation cocktail (Rotiszint eco plus, Carl Roth GmbH, Karlsruhe, Germany) was added and radioactivity was measured in a scintillation counter (*n=9* for each treatment and date). The average uptake values of three daphnids from the same Erlenmeyer flask were treated as a single replicate in order to avoid pseudo-replication. One daphnid from each treatment and date was fixed with EtOH, placed on a cover slip and dissected under a binocular microscope for later FISH and MAR-FISH analysis (see below).

Experiment II (Fig S1) was essentially performed as described for Experiment I, except that daphnids were collected from Lake Zurich, and the microbial communities in the A- and F-treatments were removed already before addition of the

radioactive tracer to assess the total potential uptake of *Daphnia sp.* epibionts. Separate triplicate sets of water samples (10mL) were used to determine tracer uptake by the pelagic bacterial assemblages. Finally, all incubations for Experiment II were performed at *in situ* (lake water) temperatures.

Staining and microscopic analysis

Dissected Daphnids on the cover slips were overlaid with a drop of 0.1% low melting point agarose and dried at 45°C. The cover slip was incubated in 90% EtOH for 45min to fix the bacterial community. FISH and MAR-FISH with probe R-BT065 (Šimek et al, 2001) (targeting bacteria affiliated with *Limnohabitans*) were essentially conducted as described before (Alonso and Pernthaler, 2005, Pernthaler et al, 2002) albeit on the cover slips, and predigestion was reduced to a lysozyme treatment of 20min only. Microscopic imaging of the filter apparatus was done on an inverse confocal laser scanning microscope (CLSM Leica SP2, Leica Microsystems, Wetzlar, Germany) at the Centre for Microscopy and Image Analysis of the University of Zurich. Images were further processed with the software package Imaris x64 version 7.5.2 (Bitplane AG, Zurich, Switzerland) and arranged using Photoshop CS5 (Adobe Systems Inc., San Jose, CA, USA).

Bacteria were counted on the filter combs of dissected and stained Lake Zurich daphnids from April 26th. Cells on a single filter comb and the corresponding appendages of seven daphnids were quantified and the result was multiplied by four (to account for the number of combs per individual).

Statistical analysis

Differences between the Rw and A treatments compared to the F treatment were determined by one-way ANOVA after LN transformation of the data to ensure normal distribution, with comparison of the means by Holm-Sidak post hoc test. The analyses were carried out using SigmaPlot 11 (Systat Software, Chicago, IL, USA).

Phylogenetic analysis

Four 16S rRNA gene clone libraries were constructed to analyse the phylogenetic composition of *Limnohabitans* bacteria on daphnids from Lake Zurich, on cultured *D. galeata* and in the surrounding media (Lake Zurich water from 5m depth and *D.*

galeata cultivation medium). For this purpose, daphnids and water samples from Lake Zurich were obtained on March 31. All daphnids were washed 3 times in sterile, UV-treated deionized water. DNA was isolated from approximately 13-16 daphnids or 25mL of filtered water using the PowerSoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). 16S rRNA gene sequences affiliated with *Lhb* were amplified using the R-Bt065 oligonucleotide (Šimek et al, 2001) as a forward primer (position: 065) and GM4r (position: 1492), a general bacterial reverse primer (Muyzer et al, 1995) resulting in a fragment of 1442 bp. PCR conditions were tested with *Limnohabitans planktonicus* strain II-D5 (Kasalicky et al, 2010), and were optimized in a reaction volume of 25µl GoTaq Green Master Mix (Promega Co., Madison WI, USA) as follows: 94°C for 300s, 94°C for 60s, 57C° for 30s, 72°C for 120s (step 2-4 repeated 30times), 72°C for 600s. Three purified PCR products of each sample were pooled and cloned into competent *Escherichia coli* cells, according to the manufacturer's protocol (pGEM-T Easy Vectors, Promega Co.). 120 clones of each transformation were picked and screened for inserts and positive clones were sequenced using the primers listed above plus primer GM1f (position: 518, Muyzer et al 1993). The sequences were assembled using DNA Baser Sequence Assembler (Heracle BioSoft S.R.L., Pitesti, Romania), aligned with the SINA web aligner (Pruesse et al, 2007) and merged into the SILVA SSU reference database 110 using the software package ARB (Ludwig et al, 2004). Uchime (Edgar et al, 2011) was used to exclude chimeric sequences. Bootstrapped Maximum Likelihood trees (1000 repetitions, (Stamatakis et al, 2008)) were calculated that comprised the sequences from this study, *Limnohabitans* sp. isolates as published in Kasalický et al (2013), and *Limnohabitans* sequences obtained from *D. magna* gut analysis (Freese and Schink, 2011). The same set of sequences was clustered into OTUs (99% identity) using Mothur (Schloss et al, 2009), and the OTU clustering pattern was compared to the results of the phylogenetic analysis. All sequences from this study are deposited in the EMBL database with accession numbers HF96498 – HF968621.

Results & Discussion

Uptake of dissolved leucine by epibionts of Daphnia galeata

We labelled a natural microbial community with tritiated leucine, and let *D. galeata* feed on it for 1h (experimental setup: Fig 1). The amount of radiolabel in *D. galeata* due to their incorporation of both, dissolved leucine and microbes (Raw water-

212 treatment [Rw], Fig 1) was compared to the uptake of dissolved leucine only, in a
213 treatment where daphnids were placed into the sample after removing the labelled
214 microbial cells by filtration (treatment Filtrate [F], Fig 1). The amount incorporated by
215 additionally feeding on microorganisms did not significantly exceed the uptake of
216 substrate from the dissolved fraction only (Fig 2), indicating that leucine-incorporating
217 heterotrophic microbes were of minor importance as a food source for daphnids
218 (Martin-Creuzburg et al, 2011, Nagata and Okamoto, 1988, Peterson et al, 1978).
219 Substantial amounts of radioactivity were detected in daphnids maintained on the
220 filtrate, suggesting uptake of dissolved substrate by the animals (Fig 2): Free-living
221 bacteria in 1 mL of water incorporated 0.1 ± 0.04 pmol leucine h^{-1} whereas three
222 times higher uptake rates (0.3 ± 0.04 pmol h^{-1}) were observed per individual daphnid
223 (Fig 2). The level of incorporation of dissolved leucine in this study far exceeds the
224 previously reported uptake of custom labelled algal exudates by daphnids (Speas
225 and Duffy, 1998). This might be ascribed to methodological issues, e.g., a low
226 labelling efficiency of the exudates.

227 To further explore the notion that leucine was readily taken up by epibiotic
228 bacteria, labelled *D. galeata* individuals were dissected and the separated pieces
229 were overlaid with a photographic emulsion, to microscopically localise the deposition
230 of radioactivity on the animal and in bacterial cells by microautoradiography (MAR)
231 and catalysed reporter deposition fluorescence *in situ* hybridization (CARD-FISH,
232 Alonso and Pernthaler 2005). Strongest labelling was detected around the trunk
233 limbs; particularly on the setae and appendages of trunk limbs 3 and 4 (TL3&4, Fig
234 3). Most uptake on these body surfaces could be assigned to single bacterial cells
235 (Fig 3). The setae and setula of trunk limb 3 and 4 serve as food capturing filter
236 sieves (e.g. Fryer 1991), and daphnids incessantly filter water through these
237 structures. Besides protecting from protistan grazers and abiotic stressors (Tang et
238 al, 2010, Tang et al, 2011), a steady supply of organic carbon and nutrients may
239 render the filter apparatus an ideal habitat for epibiotic bacteria. This interpretation is
240 further supported by our finding that almost no label was incorporated when daphnids
241 were anesthetised before being placed in the filtrate (treatment Anesthetised [A], Fig
242 1). Thus, active filtration by the animals seemed to be a prerequisite for substrate
243 uptake by the epibionts. Furthermore, the proportions of incorporated radioactivity in
244 the external body parts of dissected daphnids were between 4 and >80 times higher
245 (mean, 29.5) then in the colons (data not depicted). Epibiotic bacteria have also been

described from the feeding appendages of marine copepods, as well as on setae of other crustaceans such as the deep sea Yeti crab, *Kiwa hirsuta* (Carman and Dobbs, 1997, Goffredi et al, 2008). It should be noted that approximately 20% of the filter combs in 20 analysed daphnids were nearly uncolonized by bacteria. This might be due to molting processes, which have been shown to reduce the parasite loads on daphnids (Duneau and Ebert, 2012) and thus likely also affect the densities of other epibionts and the substrate uptake.

Active epibionts affiliated with Limnohabitans

Previous studies centred on the identity of *Daphnia* associated microbes hint at the importance of *Betaproteobacteria,* in particular of bacteria related to the genus *Limnohabitans* (Freese and Schink, 2011, Peter and Sommaruga, 2008, Qi et al, 2009). Therefore, we performed FISH on dissected individuals of *D. galeata,* using an oligonucleotide probe for a phylogenetic cluster (*Lhb*) that includes the type strains *L. planktonicus* and *L. parvus* (Šimek et al, 2001). A large proportion of the epibionts on the *D. galeata* filter apparatus were affiliated with *Lhb* (Fig 3), whereas <0.5% of cells in the surrounding cultivation water were hybridised with this probe. Most of the epibiotic *Lhb* bacteria showed visible incorporation of leucine (Fig 3), as detected by MAR-FISH (27). Planktonic *Limnohabitans* spp. are known to readily incorporate this substrate (Horňák et al, 2006, Salcher et al, 2013). Cultures of *L. planktonicus* have, moreover, been shown to profit from the presence of algae, which has been ascribed to their utilization of algal exudates (Šimek et al, 2011). In addition to the advantageous supply of such fresh DOC to *Limnohabitans* spp. on *Daphnia* filter combs by the filtration activity itself, it is also conceivable that these bacteria might further profit from the products of 'sloppy feeding', i.e. organic compounds released by the physical breaking of algal cells (e.g. Riemann et al 1986, Carman 1994).

FISH on segments of various dissected *daphnids* from Lake Zurich confirmed the presence of epibiotic *Lhb* bacteria on natural zooplankton populations (Fig. 3). While only up to 3% of the heterotrophic bacteria in lake water were affiliated with *Lhb* (Fig 5), filter combs of *daphnids* were typically covered by bacteria from this genus that were, moreover, visibly incorporating leucine. In addition, there were clear

morphological differences between planktonic *Lhb* and those associated with daphnids, e.g., only the latter formed filamentous morphotypes (Fig 3). Similar to observations in cultures of *D. galeata,* some filter combs of daphnids in Lake Zurich were also virtually free of bacteria (in 3 out of 17 analysed individuals). In addition, other, unidentified bacteria were occasionally found to dominate on the filter seata (Fig 3).

Indications for a core group of epibiontic Limnohabitans *sp.*

To investigate the phylogenetic relationship of *Lhb* bacteria on daphnids from various sources we constructed 16S ribosomal DNA clone libraries using the sequence of the *Lhb* probe as a forward primer together with a general bacterial revers primer. By this we identified bacteria associated with *D. galeata* and Lake Zurich daphnids as well as from the respective surrounding water.

No sequence obtained from the cultivation water of *D. galeata* were from the genus *Limnohabitans*. In contrast, *Lhb* sequences were obtained from water samples of Lake Zurich. These sequences were, however, considerably different from the ones retrieved from daphnids (Fig 4). These findings indicate a degree of specificity of the associations between *Lhb* bacteria and their host (Wahl et al, 2012).

Highest similarity was detected between *Lhb* sequences retrieved from daphnids from Lake Zurich and from the cultured *D. galeata* (Fig 4). The sequences clustered in two shared operational taxonomic units (OTUs, 99% identity level), as well as forming one specific OTU per source population. One of the shared clusters also included sequences retrieved from the digestive tract of *D. magna* (Freese and Schink, 2011) and the type strain *L. planktonicus* (Fig 4, Hahn et al 2010). It thus seems that there are core phylotypes closely affiliated with *L. planktonicus* that are commonly associated with different species of daphnids from various habitats, as well as a more variable set of other microbiota (Grossart et al, 2009). *L. planktonicus* was originally isolated from pelagic samples of a freshwater reservoir (Kasalicky et al, 2010). Subsequent analysis, however, revealed, that this species is not common in the pelagic zone of lacustrine waters (Jezbera et al, 2013). It is thus possible that these bacteria in fact predominately inhabit an epibiotic niche. Epibiosis may however not be the exclusive place of occurrence for a particular genotype: The

human pathogen *Vibrio cholera* is present in high abundances in the mouth area of marine copepods, but these bacteria are also found free-living in costal marine waters, albeit at low densities (Cottingham et al, 2003, Heidelberg et al, 2002, Huq et al, 1983). Thus, *V. cholera* may be part of the 'rare biosphere' within pelagic communities while abundant on zooplankton. A similar occurrence pattern might be hypothesized for bacteria related to *L. planktonicus*.

Interestingly, closely related *Lhb* sequences were found on the filter combs of *D. galeata* and in the *D. magna* digestive tract. A possible explanation lies in the feeding physiology of *Daphnia*, i.e. part of the *Lhb* population on the filter apparatus might be ingested and transported into the digestive system. Freshwater bacteria have been observed to pass the gut of daphnids alive (King et al, 1991). Whether or not *Lhb* bacteria play a role in the digestion processes within the colon of the animals remains to be explored, as well as the mode of interaction between the Daphnids and *Lhb*-epibionts on the filter combs. It should be emphasized that dead *D. galeata* were never found to be inhabited by *Lhb* bacteria (*n=6 inspected individuals*), as has already been observed for *Daphnia magna* (Freese and Schink, 2011). This may indicate that the interaction between *Lhb* bacteria and the animals is not of a pathogenic nature. Moreover, *Lhb* bacteria might actively leave the surface of *Daphnia* when the animals molt or die, as has been described for protistan epibionts (Bickel et al, 2012, Willey and Threlkeld, 1995).

Activity of Daphnia *epibionts in Lake Zurich: implication for food webs*

In order to assess the *in situ* relevance of DOC uptake by daphnids (or their epibionts, respectively) we sampled Lake Zurich throughout April and May, starting at the onset of the clear water phase after the spring phytoplankton bloom. *Daphnia* reached abundances of up to 13 adult and 28 juvenile individuals l^{-1} (Fig 5), and were dominated by members of the *D. longispina* species complex (*D. galeata, cucullata, longispina*, and hybrids; data: water supply Zurich). The uptake of dissolved leucine by daphnids and by bacterioplankton was determined on four dates (Fig 5 & 6). These experiments were in principle designed as described for the *D. galeata* cultures, with minor modifications (see Fig S1). On three of the four dates we additionally tested for uptake of NAG (Fig 6), a substrate that is not incorporated by

342 *Lhb* (Eckert et al, 2012). High uptake rates of both, dissolved leucine and NAG were
343 detected, albeit with large variations between dates (Fig 6). Interestingly, the
344 proportional amount of NAG taken up by the daphnids via consumption of labelled
345 bacteria (normalized to the labelled bacterioplankton community, i.e., (Rw-F)/B) was
346 consistently higher by about fivefold then of leucine, indicating that *Daphnia*
347 preferentially fed on NAG- rather than leucine-incorporating bacteria. This might be
348 explained by the high NAG uptake rates of large filamentous bacteria that are more
349 likely to be grazed by daphnids than, e.g. small rod-shaped cells specialized for
350 leucine incorporation (Eckert et al, 2013, Kragelund et al, 2008, Langenheder and
351 Jürgens, 2001, Pernthaler et al, 2004). Alternatively, there might be NAG uptake by
352 some phytoplankton species that are consumed by daphnids (Nedoma et al, 1994).
353 Thus the transfer mode of DOC to daphnids may in fact differ for individual organic
354 compounds: While the direct incorporation of heterotrophic bacteria seemed
355 unimportant for leucine uptake of zooplankton, their foraging on NAG-labelled
356 microbes was of greater relevance.

357 The observed uptake of both, NAG and leucine by daphnids incubated in
358 bacteria-free filtrates is evidence for a high, temporarily variable DOC incorporation *in*
359 *situ*, likely mediated via the attached bacterial flora. This is suggested by the high
360 proportion of tracer incorporation on the external body surfaces, as compared to the
361 digestive tract (data not depicted). Each daphnid approximately hosted 10^5 bacteria
362 on their filter combs, as estimated from counts on individuals from April 26. Assuming
363 that 50-60% of the tracer is incorporated on these surfaces (as concluded from our
364 laboratory experiment, data not depicted), the per cell activity of bacteria on the filter
365 combs is more than two orders of magnitude higher than of free-living lake bacteria.
366 This is in agreement with findings from marine systems that zooplankton associated
367 bacteria are metabolically more active than free-living bacteria (Møller et al, 2007).
368 Moreover, attached bacteria move through the water column with the host (Grossart
369 et al, 2010). Daphnids tend to search for patches with higher food concentration
370 (Dodson et al, 1997, Larsson and Kleiven, 1996) and might thereby give an
371 additional advantage to attached bacteria, i.e., by transporting them to hot-spots of
372 organic carbon and inorganic nutrients (Grossart et al, 2010).

373 If extrapolated to the total daphnid population in lake water, the incorporation
374 amounted to up to 8% of the leucine and nearly 9% of the NAG uptake by the

bacterioplankton. While this proportion may seem small at a first glance, such direct transfer of low molecular weight DOC to zooplankton may nevertheless be of considerable importance. The positive effect of *Daphnia* on the size of fish populations in lakes is mainly attributed to the fact that *Daphnia* feed on primary producers and are in turn consumed by fish i.e. the cascade algae-daphnia-fish is considered to be highly efficient because of its shortness (Stockner and Porter, 1988, Stockner and Shortreed, 1989). Similarly, the prokaryotic epibionts on daphnids may form a more direct link between microbial DOC uptake and fish predation. Such a shortcut would circumvent the passage of substrates through intermediate levels of the microbial food web, thereby avoiding the significant respiration losses associated with these trophic transition (Lindeman, 1942, Pomeroy and Wiebe, 1988, Stockner and Shortreed, 1989). This may be illustrated by a highly simplified lake food web consisting of planktivorous fish, *Daphnia*, phytoplankton, protists, bacteria, and DOC. In this food web, fish feed on *Daphnia* that feed on protists and phytoplankton. The latter provides DOC for bacteria that are in turn incorporated by protists. However, the pathway of the transfer of organic carbon changes when epibiosis is included: If fish feed on Daphnia, they also ingest the attached bacteria. Thus, fish concomitantly consume organisms that are considered to belong to different trophic levels, and all of these organisms will contribute to their biomass. Considering the '*Rule of 10'* concept (stating that only 10% of energy is transferred from a given trophic level to the next one (Lindeman, 1942)), at least 0.1% of the total microbial DOC (leucine) uptake would be transferred to fish via the epibiotic bacteria. In comparison, less than 0.01% of DOC would reach fish through the 'classical' microbial loop due to the losses that occur across two or more intermediate trophic levels (i.e., protists, daphnids). Thus, ten times more carbon would be transferred to fish from epibiotic bacteria than from free-living bacteria. This suggests that zooplankton epibionts such as *Limnohabitans* sp. might play a disproportionally important role for the transfer of DOC from both, autochthonous and terrestrial sources to the top trophic levels in lacustrine ecosystems.

Acknowledgements

We are particularly indebted to Piet Spaaks, Marina Manca and Esther Keller for providing us with *Daphnia galeata* and the introduction to *Daphnia* handling, and to Julia Huber for Daphnia artwork. Michaela Salcher is acknowledged for providing DNA from Lake Zurich, Jörg Villiger for bioinformatic assistance, Eugen Loher for help with sampling and Thomas Posch for comments on data analysis and the manuscript. We also wish to thank Karel Šimek and Voijta Kasalický for interesting discussions about *Limnohabitans*. The assistance and support of the Centre for Microscopy and Image Analysis at the University of Zurich is acknowledged. This study was funded by the Swiss National Science Foundation (Project 31EE30-132771) as part of the European Collaborative Research Program (EUROCORES) Ecological and Evolutionary Functional Genomics (EuroEEFG) of the European Science Foundation.

Supplementary information is available at ISMEJ's website. The authors state that there are no conflicts of interests.

References

Alonso C, Pernthaler J (2005). Incorporation of glucose under anoxic conditions by bacterioplankton from coastal North Sea surface waters. *Appl Environ Microbiol* **71:** 1709-1716.

Bascompte J (2010). Structure and Dynamics of Ecological Networks. *Science* **329:** 765-766.

Beier S, Bertilsson S (2013). Bacterial chitin degradation—mechanisms and ecophysiological strategies. *Frontiers in Microbiology* **4**.

Bickel SL, Tang KW, Grossart H-P (2012). Ciliate epibionts associated with crustacean zooplankton in German lakes: Distribution, motility and bacterivory. *Frontiers in Microbiology* **3**.

Brett MT, Kainz MJ, Taipale SJ, Seshan H (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proc Natl Acad Sci USA* **106:** 21197-21201.

Carman KR (1994). Stimulation of marine free-living and epibiotic bacterial activity by copepod excretions. *FEMS Microbiol Ecol* **14:** 255-261.

Carman KR, Dobbs FC (1997). Epibiotic microorganisms on copepods and other marine crustaceans. *Microsc Res Tech* **37:** 116-135.

Cole JJ, Carpenter SR, Pace ML, Van de Bogert MC, Kitchell JL, Hodgson JR (2006). Differential support of lake food webs by three types of terrestrial organic carbon. *Ecol Lett* **9:** 558-568.

Corno G, Villiger J, Pernthaler J (2012). Coaggregation in a microbial predator–prey system affects competition and trophic transfer efficiency. *Ecology* **94:** 870-881.

Cottingham KL, Chiavelli DA, Taylor RK (2003). Environmental microbe and human pathogen: the ecology and microbiology of Vibrio cholerae. *Frontiers in Ecology and the Environment* **1:** 80-86.

del Giorgio PA, Cole JJ (1998). Bacterial growth efficiency in natural aquatic systems. *Annu Rev Ecol Syst* **29:** 503-541.

Dodson SI, Ryan S, Tollrian R, Lampert W (1997). Individual swimming behavior of Daphnia: effects of food, light and container size in four clones. *J Plankton Res* **19:** 1537-1552.

Duneau D, Ebert D (2012). The role of moulting in parasite defence. *Proc R Soc B-Biol Sci* **279:** 3049-3054.

Ebert D (2008). Host-parasite coevolution: Insights from the Daphnia-parasite model system. *Curr Opin Microbiol* **11:** 290-301.

Eckert EM, Salcher MM, Posch T, Eugster B, Pernthaler J (2012). Rapid successions affect microbial N-acetyl-glucosamine uptake patterns during a lacustrine spring phytoplankton bloom. *Environ Microbiol* **14:** 794-806.

Eckert EM, Baumgartner M, Huber IM, Pernthaler J (2013). Grazing resistant freshwater bacteria profit from chitin and cell-wall-derived organic carbon. *Environ Microbiol* **15:** 2019-2030.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27:** 2194-2200.

Freese H, Eggert A, Garland J, Schumann R (2009). Substrate Utilization Profiles of Bacterial Strains in Plankton from the River Warnow, a Humic and Eutrophic River in North Germany. *Microb Ecol* **Epub**.

Freese H, Schink B (2011). Composition and Stability of the Microbial Community inside the Digestive Tract of the Aquatic Crustacean *Daphnia magna*. *Microb Ecol* **62:** 882-894.

Fryer G (1991). Functional Morphology and the Adaptive Radiation of the Daphniidae (Branchiopoda: Anomopoda). *Phil Trans R Soc Lond B* **331:** 1-99.

484 Goffredi SK, Jones WJ, Erhlich H, Springer A, Vrijenhoek RC (2008). Epibiotic bacteria associated with
485 the recently discovered Yeti crab, Kiwa hirsuta. *Environ Microbiol* **10:** 2623-2634.

486
487 Grey J, Jones RI, Sleep D (2001). Seasonal changes in the importance of the source of organic matter
488 to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnol Oceanogr* **46:**
489 505-513.

490
491 Grossart HP, Dziallas C, Tang KW (2009). Bacterial diversity associated with freshwater zooplankton.
492 *Environ Microbiol Rep* **1:** 50-55.

493
494 Grossart HP, Dziallas C, Leunert F, Tang KW (2010). Bacteria dispersal by hitchhiking on zooplankton.
495 *Proc Natl Acad Sci USA* **107:** 11959-11964.

496
497 Hahn MW, Kasalický V, Jezbera J, Brandt U, Jezberova J, Šimek K (2010). Limnohabitans curvus gen.
498 nov., sp nov., a planktonic bacterium isolated from a freshwater lake. *Int J Syst Evol Microbiol* **60:**
499 1358-1365.

500
501 Heidelberg JF, Heidelberg KB, Colwell RR (2002). Seasonality of Chesapeake Bay bacterioplankton
502 species. *Appl Environ Microbiol* **68:** 5488-5497.

503
504 Horňák K, Jezbera J, Nedoma J, Gasol JM, Šimek K (2006). Effects of resource availability and
505 bacterivory on leucine incorporation in different groups of freshwater bacterioplankton, assessed
506 using microautoradiography. *Aquat Microb Ecol* **45:** 277-289.

507
508 Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR (1983). Ecological relationships between
509 Vibrio cholerae and planktonic crustacean copepods. *Appl Environ Microbiol* **45:** 275-283.

510
511 Jezbera J, Jezberová J, Kasalický V, Šimek K, Hahn MW (2013). Patterns of *Limnohabitans*
512 Microdiversity across a Large Set of Freshwater Habitats as Revealed by Reverse Line Blot
513 Hybridization. *PLoS One* **8:** e58527.

514
515 Jørgensen NOG (1992). Incorporation of [3H] leucine and [3H] valine into protein of freshwater
516 bacteria: Uptake kinetics and intracellular isotope dilution. *Appl Environ Microbiol* **58:** 3638-3646.

517
518 Kasalicky V, Jezbera J, Simek K, Hahn MW (2010). Limnohabitans planktonicus sp. nov. and
519 Limnohabitans parvus sp. nov., planktonic betaproteobacteria isolated from a freshwater reservoir,
520 and emended description of the genus Limnohabitans. *Int J Syst Evol Microbiol* **60:** 2710-2714.

521
522 Kasalický V, Jezbera J, Hahn MW, Šimek K (2013). The Diversity of the *Limnohabitans* Genus, an
523 Important Group of Freshwater Bacterioplankton, by Characterization of 35 Isolated Strains. *PLoS*
524 *One* **8:** e58209.

525
526 Kerb H (1911). Über den Nährwert der im Wasser gelösten Stoffe. *Internationale Revue der gesamten*
527 *Hydrobiologie und Hydrographie* **3:** 496-505.

528
529 King CH, Sanders RW, Shotts EB, Porter KG (1991). Differential survival of bacteria ingested by
530 zooplankton from a stratified eutrophic lake. *Limnol Oceanogr* **36:** 829-845.
531
532 Kirchman D, Knees E, Hodson R (1985). Leucine Incorporation and its Potenial as a Measure of
533 Protein-Sythesis by Bacteria in Natural Aquatic Systems. *Appl Environ Microbiol* **49:** 599-607.
534
535 Köllner KE, Carstens D, Keller E, Vazquez F, Schubert CJ, Zeyer J *et al* (2012). Bacterial Chitin
536 Hydrolysis in Two Lakes with Contrasting Trophic Statuses. *Appl Environ Microbiol* **78:** 695-704.
537
538 Kragelund C, Levantesi C, Borger A, Thelen K, Eikelboom D, Tandoi V *et al* (2008). Identity, abundance
539 and ecophysiology of filamentous bacteria belonging to the Bacteroidetes present in activated sludge
540 plants. *Microbiology-(UK)* **154:** 886-894.
541
542 Krogh A (1930). Über die Bedeutung von gelösten organischen Substanzen bei der Ernährung von
543 Wassertieren. *Zeitschrift für vergleichende Physiologie* **12:** 668-681.
544
545 Kubitschek H (1968). Constancy of Uptake During the Cell Cycle in Escherichia coli. *Biophysical Journal*
546 **8:** 1401-1412.
547
548 Kuwae T, Miyoshi E, Hosokawa S, Ichimi K, Hosoya J, Amano T *et al* (2012). Variable and complex
549 food web structures revealed by exploring missing trophic links between birds and biofilm. *Ecol Lett*
550 **15:** 347-356.
551
552 Langenheder S, Jürgens K (2001). Regulation of bacterial biomass and community structure by
553 metazoan and protozoan predation. *Limnol Oceanogr* **46:** 121-134.
554
555 Larsson P, Kleiven O (1996). Food search and swimming speed in. *Zoooplankton: Sensory Ecology and*
556 *Physiology, Gordon & Breach, Amsterdam, the Netherlands***:** 375-387.
557
558 Lindeman RL (1942). The trophic-dynamic aspect of ecology. *Ecology* **23:** 399-418.
559
560 Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar *et al* (2004). ARB: a software
561 environment for sequence data. *Nucleic Acids Res* **32:** 1363-1371.
562
563 Martin-Creuzburg D, Beck B, Freese HM (2011). Food quality of heterotrophic bacteria for Daphnia
564 magna: evidence for a limitation by sterols. *FEMS Microbiol Ecol* **76:** 592-601.
565
566 Miner BE, De Meester L, Pfrender ME, Lampert W, Hairston NG (2012). Linking genes to communities
567 and ecosystems: Daphnia as an ecogenomic model. *Proc R Soc B-Biol Sci* **279:** 1873-1882.
568
569 Møller EF, Riemann L, Søndergaard M (2007). Bacteria associated with copepods: abundance, activity
570 and community composition. *Aquat Microb Ecol* **47:** 99-106.
571

572 Mougi A, Kondoh M (2012). Diversity of Interaction Types and Ecological Community Stability.
573 *Science* **337:** 349-351.

574
575 Muyzer G, De Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by
576 denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding
577 for 16S rRNA. *Appl Environ Microbiol* **59:** 695-700.

578
579 Muyzer G, Teske A, Wirsen CO, Jannasch HW (1995). Phylogenetic relationships of Thiomicrospira
580 species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel
581 electrophoresis of 16S rDNA fragments. *Arch Microbiol* **164:** 165-172.

582
583 Nagata T, Okamoto K (1988). Filtering rates on natural bacteria by Daphnia longispina and
584 Eodiaptomus japonicus in Lake Biwa *J Plankton Res* **10:** 835-850.

585
586 Nedoma J, Vrba J, Hejzlar J, Šimek K, Straskrabová V (1994). N-Acetylglucosamine Dynamics in Fresh-
587 Warer Environments - Concentration of Amino-Sugars, Extracellular Enzyme-Activities, and Microbial
588 Uptake. *Limnol Oceanogr* **39:** 1088-1100.

589
590 Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011). A Guide to the Natural History of
591 Freshwater Lake Bacteria. *Microbiol Mol Biol Rev* **75:** 14-49.

592
593 Pace ML, Cole JJ, Carpenter SR, Kitchell JF, Hodgson JR, Van de Bogert MC *et al* (2004). Whole-lake
594 carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* **427:** 240-243.

595
596 Pernthaler A, Pernthaler J, Amann R (2002). Fluorescence in situ hybridization and catalyzed reporter
597 deposition for the identification of marine bacteria. *Appl Environ Microbiol* **68:** 3094-3101.

598
599 Pernthaler J, Zöllner E, Warnecke F, Jürgens K (2004). Bloom of filamentous bacteria in a mesotrophic
600 lake: Identity and potential controlling mechanism. *Appl Environ Microbiol* **70:** 6272-6281.

601
602 Peter H, Sommaruga R (2008). An evaluation of methods to study the gut bacterial community
603 composition of freshwater zooplankton. *J Plankton Res* **30:** 997-1006.

604
605 Peterson BJ, Hobbie JE, Haney JF (1978). Daphnia grazing on natural bacterial. *Limnol Oceanogr* **23:**
606 1039-1044.

607
608 Pomeroy L, Wiebe W (1988). Energetics of microbial food webs. *Hydrobiologia* **159:** 7-18.

609
610 Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig WG, Peplies J *et al* (2007). SILVA: a comprehensive
611 online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB.
612 *Nucleic Acids Res* **35:** 7188-7196.

613
614 Qi WH, Nong G, Preston JF, Ben-Ami F, Ebert D (2009). Comparative metagenomics of Daphnia
615 symbionts. *BMC Genomics* **10:** 1471-2164.

Riemann B, Jørgensen NOG, Lampert W, Fuhrman JA (1986). Zooplankton Induced Changes in Dissolved Free Amino Acids and in Production Rates of Freshwater Bacteria. *Microb Ecol* **12:** 247-258.

Salcher MM, Posch T, Pernthaler J (2013). In situ substrate preferences of abundant bacterioplankton populations in a prealpine freshwater lake. *Isme J* **7:** 896-907.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al* (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl Environ Microbiol* **75:** 7537-7541.

Šimek K, Pernthaler J, Weinbauer MG, Horňák K, Dolan JR, Nedoma J *et al* (2001). Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Appl Environ Microbiol* **67:** 2723-2733.

Šimek K, Horňák K, Jezbera J, Mašin M, Nedoma J, Gasol JM *et al* (2005). Influence of top-down and bottom-up manipulations on the R-BT065 subcluster of beta-proteobacteria, an abundant group in bacterioplankton of a freshwater reservoir. *Appl Environ Microbiol* **71:** 2381-2390.

Šimek K, Kasalický V, Zapomelova E, Horňák K (2011). Alga-Derived Substrates Select for Distinct Betaproteobacterial Lineages and Contribute to Niche Separation in Limnohabitans Strains. *Appl Environ Microbiol* **77:** 7307-7315.

Speas DW, Duffy WG (1998). Uptake of dissolved organic carbon (DOC) by Daphnia pulex. *J Freshw Ecol* **13:** 457-463.

Stamatakis A, Hoover P, Rougemont J (2008). A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Syst Biol* **57:** 758-771.

Stockner J, Porter K (1988). Microbial Food Webs in Freshwater Planktonic Ecosystems. In: Carpenter SR (ed). *Complex Interactions in Lake Communities*. Springer New York. pp 69-83.

Stockner JG, Shortreed KS (1989). Algal picoplankton production and contribution to food-webs in oligotrophic British Columbia lakes. *Hydrobiologia* **173:** 151-166.

Taipale S, Kankaala P, Tiirola M, Jones RI (2008). Whole-lake dissolved inorganic (13)C additions reveal seasonal shifts in zooplankton diet. *Ecology* **89:** 463-474.

Tang KW, Turk V, Grossart HP (2010). Linkage between crustacean zooplankton and aquatic bacteria. *Aquat Microb Ecol* **61:** 261-277.

Tang KW, Dziallas C, Grossart HP (2011). Zooplankton and aggregates as refuge for aquatic bacteria: protection from UV, heat and ozone stresses used for water treatment. *Environ Microbiol* **13:** 378-390.

Thébault E, Fontaine C (2010). Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks. *Science* **329:** 853-856.

Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F (2012). The second skin: Ecological role of epibiotic biofilms on marine organisms. *Frontiers in Microbiology* **3:** 1-21.

Willey RL, Threlkeld ST (1995). Changes in epibiotic burden during the intermolt of Daphnia determined by hypodermal retraction stages. *Cladocera as Model Organisms in Biology*. Springer. pp 213-219.

Figure Legends

Figure 1: Schematic depiction of the setup of experiment I. For the raw water (Rw) and filtrate (F) treatments active *D. galeata* individuals were incubated for 1 h together with the lake water microbial assemblages (Rw) or in lake water filtrates (F). Both Rw and F treatments had been pre-incubated with tritiated leucine for 1 h before the addition of daphnids. In the anesthetised treatment (A) daphnids were placed in CO_2-enriched mineral water prior to incubation in the lake water filtrates (negative control). Subsequently, daphnids were either dissected for microautoradiography (MAR-FISH, 1 animal), or total leucine uptake per individual was determined by scintillation counting (3 animals). All treatments were done in triplicates.

Figure 2: Leucine uptake by the heterotrophic bacterial community (B) after 1h of labelling, by active *Daphnia galeata* after 1h of incubation in the labelled raw water (Rw), and by active (F) and anesthetised (A) *D. galeata* maintained for 1h in tracer containing 0.2 µm prefiltered lake water. Error bars represent the standard errors of triplicate water samples (B) or the standard errors of measurements from 3 replicates (Rw, F, A). Different lowercase letters above the bars of Rw, F and A indicated significant differences between treatments (ANOVA, $p<0.5$).

694

695 *Figure 3:* Confocal photomicrographs of *Daphnia spp.* epibionts (left), and localisation
696 of the depicted structures in a schematic drawing of a daphnid (right). Green cells
697 are hybridised with probe R-Bt065, targeting *Lhb* bacteria. Depicted in red are
698 other DNA-containing objects, i.e., bacterial cells that are not *Lhb* and nuclei of
699 *Daphnia*. Panels A and B: *D. galeata* feeding appendages with hybridised *Lhb*
700 *cells*. Panel C: *D. galeata* feeding combs with hybridised *Lhb* cells surrounded by
701 black halos from microautoradiography staining that indicates the uptake of
702 tritiated leucine by these bacteria. Panel D: feeding appendage of daphnid from
703 Lake Zurich with hybridised *Lhb* and numerous other bacteria. TL3, TL4: trunk
704 limbs 3 and 4

705 *Figure 4:* Left panel: Shared and unique operational taxonomic units (OTUs, 99%
706 identity cut-off) of 16S rRNA gene sequences of *Lhb* bacteria from cultured
707 *Daphnia galeata*, from Lake Zurich daphnids, and from cultured *D. magna* (Freese
708 et al, 2009). Right panel: phylogenetic analysis (Maximum Likelihood method) of
709 *Limnohabitans* spp. including sequences from cultured strains (Kasalický et al,
710 2013) and *Daphnia* metagenome (Qi et al, 2009). The individual OTUs are
711 depicted as grey boxes, the broken line links sets of sequences from a single
712 OTU. Values in brackets refer to the numbers of sequences in 'collapsed' clusters
713 depicted as wedges. Only bootstrap values >50% (1000 replications) are reported.
714 Scale bar, 10% estimated sequence divergence.

715

716 *Figure 5:* Development of chlorophyll *a*, temperature, and of the populations of
717 *Daphnia sp.* and pelagic *Lhb* bacteria in Lake Zurich from April 17 to May 31 2013.
718 Upper panel: Chlorophyll *a* concentrations and temperature between 0 and 15m
719 depth. Grey circles indicate the dates and depth of samplings for the incubations
720 with radiolabeled tracers, and for DNA extraction to identify *Lhb* epibionts (last
721 time point). Lower panel: Abundances of juvenile and adult daphnids and
722 proportions of pelagic *Lhb* bacteria of all bacterioplankton cells.

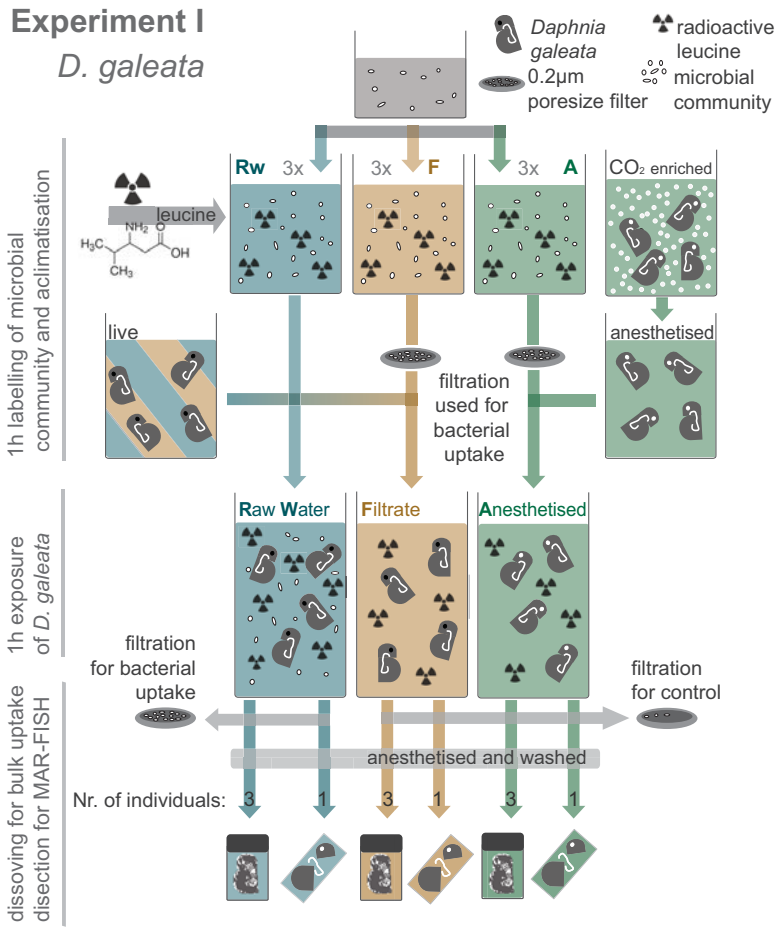
723

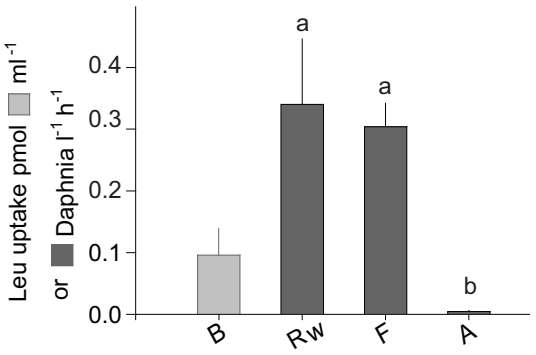
724 *Figure 6:* Uptake of tritiated leucine (upper panels) and N-acetyl glucosamine (NAG,
725 lower panels) by the heterotrophic bacterial community (B) after 1h of incubation,

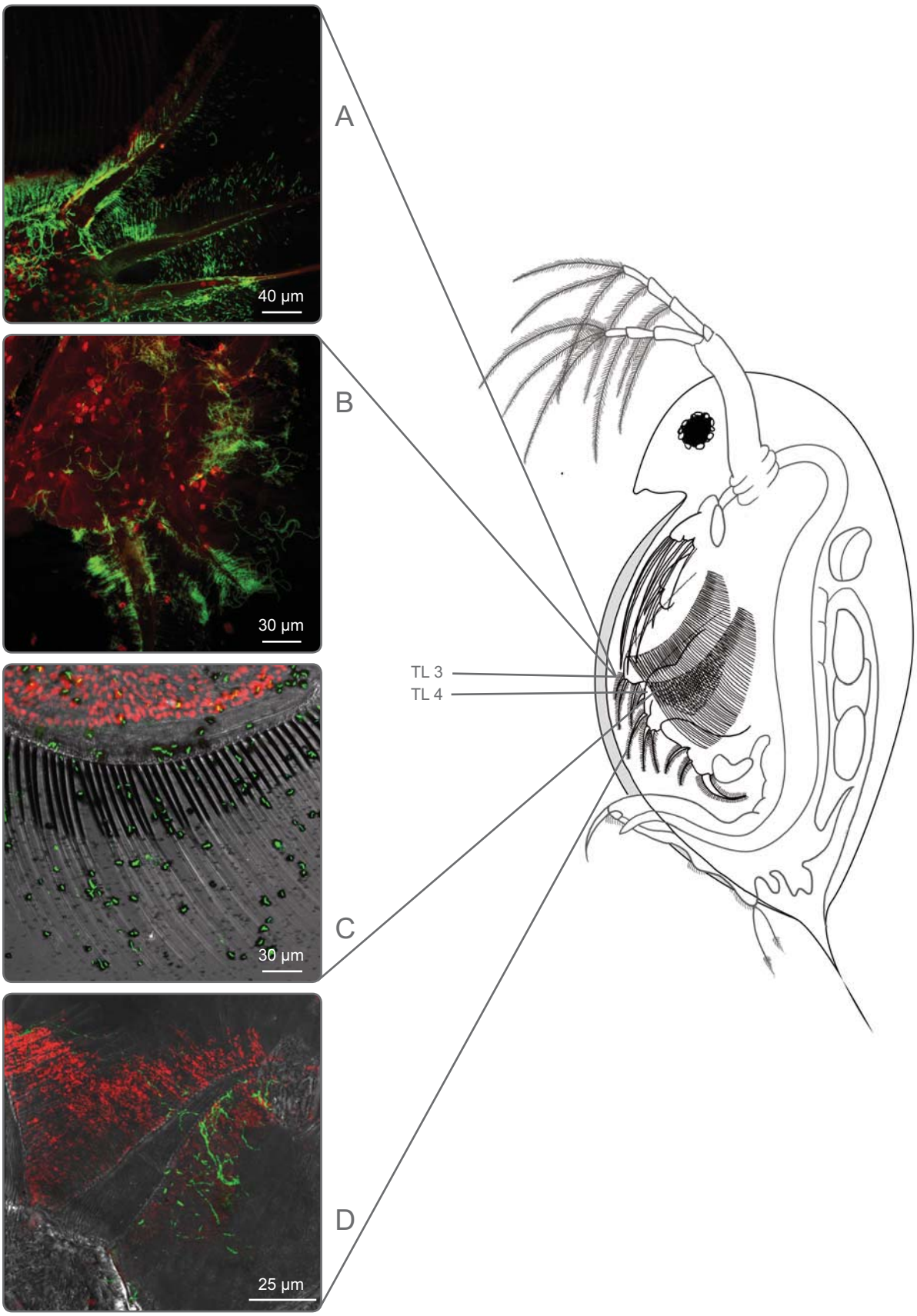
by lake daphnids after 1h of incubation in this labelled raw water (Rw), and by active (F) and anesthetised lake daphnids (A) maintained for 1h in tracer containing 0.2 µm prefiltered lake water. The experimental dates are indicated in each panel. Error bars represent the standard errors of triplicate water samples (B) or the standard errors of measurements from 3 replicates (Rw, F, A). Different lowercase letters on the bars of Rw, F and A indicated significant differences between the treatments ($p<0.5$).

Legends for Supplementary Figures:

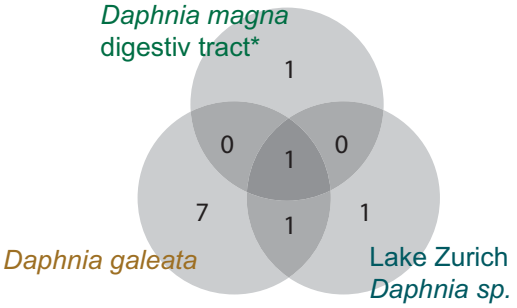
Figure S1: Schematic depiction of the setup of experiment II. For the raw water (Rw) individual *Daphnia* spp. from Lake Zurich were incubated for 1 h together with the lake water microbial assemblages (Rw), that had been pre-incubated with tritiated leucine for 1 h before the addition of daphnids. For the filtrate (F) and the anesthetised treatment (A, negative control) active and CO_2-treated *Daphnia* spp., respectively, were maintained for 1h on lake water filtrates that had been pre-incubated with leucine . Subsequently, daphnids were either dissected for microautoradiography (MAR-FISH, 1 animal), or total leucine uptake per individual was determined by scintillation counting (3 animals). Additionally, lake water samples were incubated with leucine for 1h to determine uptake of the microbial community. All treatments were done in triplicates.


Experiment I
D. galeata
Daphnia galeata
0.2µm poresize filter
radioactive leucine
microbial community
1h labelling of microbial community and aclimatisation
leucine
Rw 3x
3x F
3x A
CO2 enriched
live
anesthetised
filtration used for bacterial uptake
1h exposure of D. galeata
Raw Water
Filtrate
Anesthetised
filtration for bacterial uptake
filtration for control
anesthetised and washed
dissoving for bulk uptake disection for MAR-FISH
Nr. of individuals:
3
1
3
1
3
1


Leu uptake pmol ml-1
or Daphnia l-1 h-1
0.0
0.1
0.2
0.3
0.4
a
a
b
B
Rw
F
A

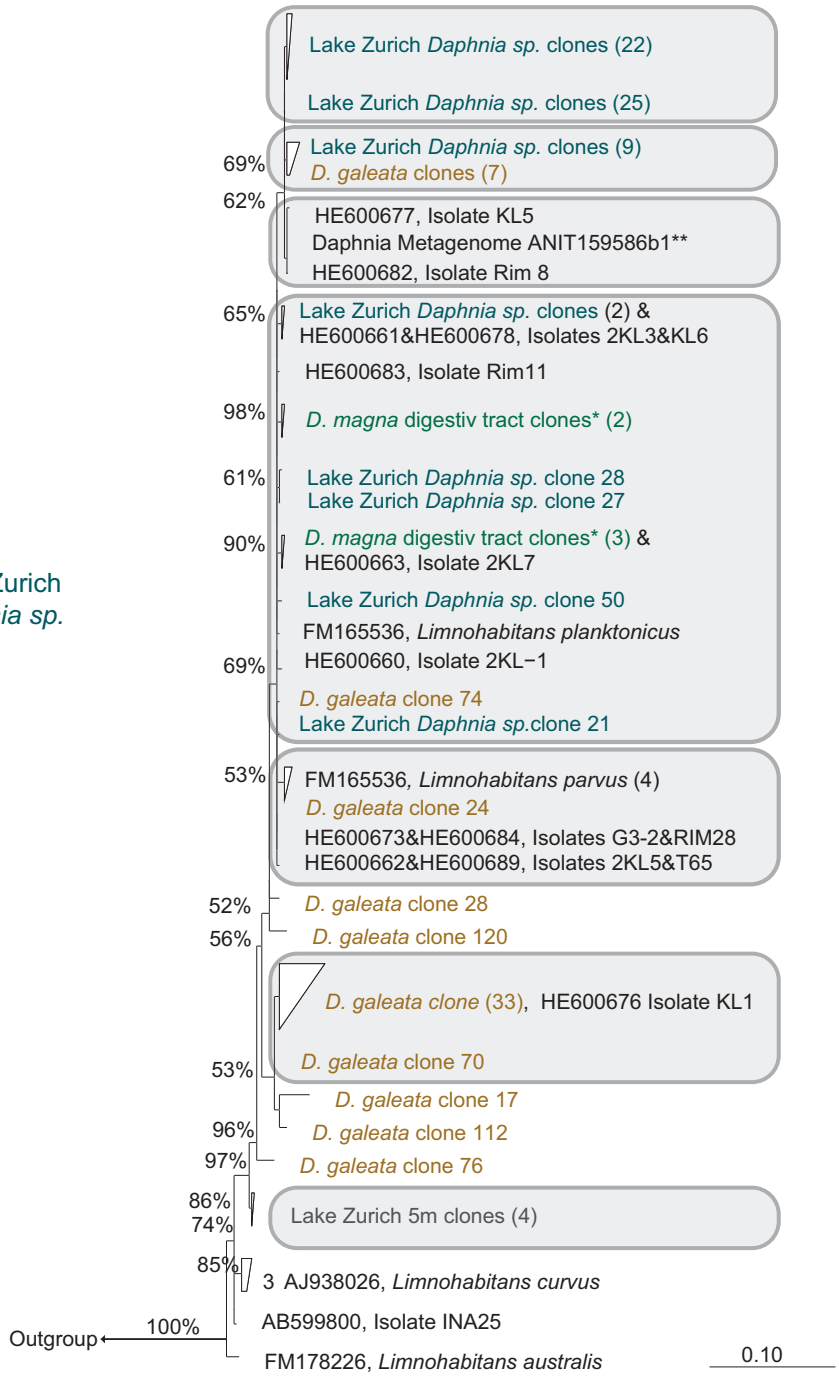

A
40 µm
B
30 µm
C
30 µm
D
25 µm
TL 3
TL 4

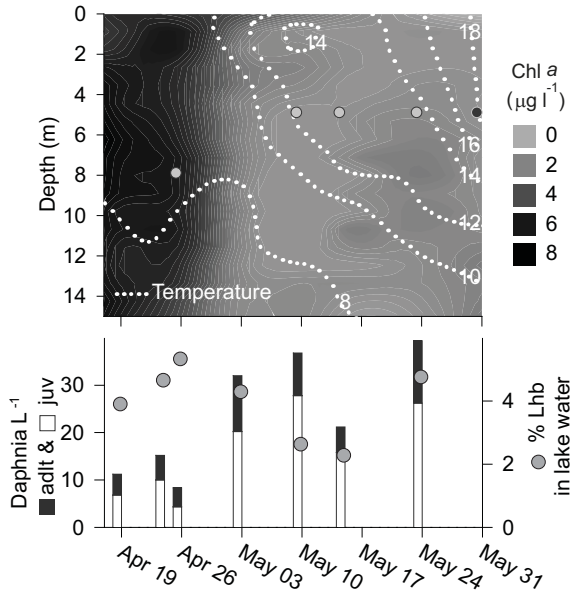
Unique and shared OTUs
(99% identity)

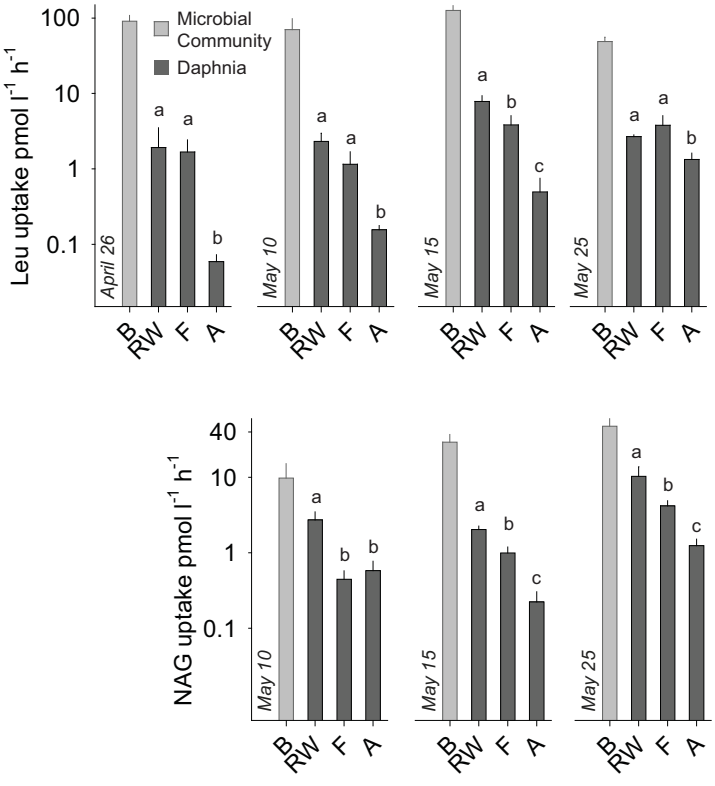


* from Freese et al. (2011)

* * from Qi et al. (2009)




Depth (m)
0
2
4
6
8
10
12
14
Temperature
18
16
14
12
10
8
Chl a
(µg l-1)
0
2
4
6
8
Daphnia L-1
adlt & juv
30
20
10
0
% Lhb
in lake water
4
2
0
Apr 19
Apr 26
May 03
May 10
May 17
May 24
May 31


Leu uptake pmol l-1 h-1
100
10
1
0.1
Microbial Community
Daphnia
April 26
May 10
May 15
May 25
a
a
b
a
a
b
a
b
c
a
a
b
B
RW
F
A
NAG uptake pmol l-1 h-1
40
10
1
0.1
May 10
May 15
May 25
a
b
b
a
b
c
a
b
c